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16 ABSTRACT

This study investigated the relation of short-term measurability of malathion and some of its metabolites in fish to poisoning of fish in the laboratory. Data indicated analysis for malathion monoacid in gut and measurement of brain acetylcholinesterase activity in fish from the natural environment are a practical measure of poisoning caused by malathion in sensitive fish. Measurable concentrations of parent compound need not be present, but acetylcholinesterase inhibition in brain must occur for fish to be poisoned.

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The Relationship of Malathion and Its Metabolities to Fish Poisoning[1]

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INTRODUCTION

Malathion is a widely used organophosphate insecticide with an annual production estimated to be in excess of 1.3×10^7 kg in the United States (ENVIRONMENTAL PROTECTION AGENCY 1977-1). It may enter surface water through surface runoff (Du...; ENVIRONMENTAL PROTECTION AGENCY 1975a,c,d) or through direct spray for mosquito control (WEIDHAAS et al. 1970, DUPREE and HALL 1971, FORNEROT 1972). Concentrations ranging from 0.05 to 100 ng malathion/l in farm surface waters have been reported (WEIDHAAS et al. 1970, DUPREE and HALL 1971, ENVIRONMENTAL PROTECTION AGENCY 1975b). But interpretation of effects of malathion on non-target species is difficult because the toxic agent during poisoning is a "persistent" metabolite bound to an enzyme in a form not identifiable by analytical chemical analysis of animal tissue (ALDRIDGE 1971, FUKUTO 1971). Poisoning results from accumulation of a neurotransmitter substance (acetylcholine) because the active site of the hydrolyzing enzyme (acetylcholinesterase) of nerve cells is phosphorylated by oxonyl or oxyl phosphate after conversion of malathion to its oxygen analog (O'BRIEN 1960, ALDRIDGE 1963, HARTLEY 1970, ALDRIDGE 1971, FUKUTO 1971). In animals from the natural environment, enzyme inhibition is measurable in nerve tissue and indicates poisoning even though chemical residues of the oxygen-bound pesticide metabolites are not measurable.

After inhibition of acetylcholinesterase, the oxygen-bound metabolites of malathion may cause inhibition for several weeks after exposure is discontinued (KILGORE 1961, CARTER 1971, HART and KEARNS 1974) and the parent compound disappears from water. Although acetylcholinesterase inhibition in animals from the environment indicates poisoning, nervous acetylcholinesterase particles are applied to the environment and the specific agent or agents causing poisoning need to be identified. To infer what parent compound caused poisoning, it may be necessary to find metabolites that are readily measurable during poisoning. In this report, we determine the relation of short-term susceptibility of malathion and some of its metabolites in fish to poisoning of fish in the laboratory. Degree of poisoning is determined by brain acetylcholinesterase inhibition and deaths in exposed populations.

[1] Gulf Breeze Contribution Number 215.

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MATERIALS AND METHODS

Exposure of fish in the laboratory: Two laboratory exposures of fish were made. The first exposure was to 75 µg malathion/l of flowing seawater. The second exposure was to 3 µg malathion/l and the third to 30 µg malathion/l. Mullet, Mugil cephalus, (20-30) mm total length) were obtained from wild fish populations and acclimated to laboratory conditions at least 2 weeks before testing. In each test, 4 replicates of 12 fish each were exposed to nominal grade malathion (93% pure) in 8-liter acrylic plastic aquaria. The malathion was dispersed in acetone and injected into the water by means of a Lab-Line[2] pump at metering jars. Solvent (acetone) never exceeded 1 ml/l of water and did not affect acetylcholinesterase activity (COPPAGE et al. 1975). Temperature ranged from 21-28°C and salinity from 21-29 parts per thousand during the tests. The fish consumed no 75 µg malathion/l were sacrificed after 24 h for routine analyses of whole body, brain, liver, flesh (muscle) and gut (whole gut with contents). In addition to 30 µg malathion/l, 3 replicate samples of fish were removed from the malathion aquaria at 0.5, 1, 4, 8, 24, 48 and 72 h for analyses of brain acetylcholinesterase and residues in gut. In the 30 µg malathion/l exposure, fish samples were removed for analyses at 1, 4, and 24 h exposure and at 24, 48, 120 and 192 h after exposure was discontinued (depuration).

Determination of enzyme activity in laboratory exposures: The acetylcholinesterase of the pooled brain and flesh samples was assayed with a recording pH stat (COPPAGE 1972). Each assay sample consisted of pooled brains taken from 3 fish. Total enzyme activity was fractional loss of activity of untreated fish taken throughout the testing period from the same populations as fish exposed to malathion. Inhibition was determined by assay of fish that survived a designated time, as percentage inhibition was determined by comparison with sustained activity. Specific enzyme activities of exposed fish were statistically compared to control activity by Student's t-test ($p < 0.05$).

Residue analyses: Methods for malathion, malaoxon, malathion monoacid (MOA), and malathion di-acid (DOA) in fish were determined (GUHA and MOORE). Tissue (brain from selected organs) was placed in a 25 x 150 mm culture tube, spiked with 37 µl dimethoate and its acid degradation product TOA. The extraction began with 10 ml acetonitrile which had been acidified with 20 µl 1 N HCl. The extraction was carried out on a Wilbur polytron for 20 sec at 20,000 rpm. After extraction, the culture tubes were centrifuged and the acidified acetonitrile decanted into 125 ml of 2% aqueous sodium sulfate. The pH was adjusted to 8 and

[2] Harvard Apparatus Co., Hillis, Mass. Mention of commercial products does not constitute endorsement by the Environmental Protection Agency.

solution and phenthoate separated by extraction twice with 25 ml petroleum ether. Malaoxon was removed by extracting the aqueous solution twice with 25 ml methylene chloride. MDA, IDA, and TDA were removed by adjusting the pH of the aqueous solution to 1, adding 25 (w/v%) solid sodium chloride, and extracting twice with 25 ml ethyl acetate. The petroleum ether and methylene chloride extracts were dried by eluting through a plug of anhydrous sodium sulfate and collected in a cryocollecting dish. The ethyl acetate extracts were dried by eluting through a plug of acidified sodium sulfate, collected in a cryocollecting dish, and methylated with diazomethane as described by SCHOR and KILLINGER (1968).

The extracts were concentrated by placing the cryocollecting dish in a heat on a dilute water at 35°C and passing a gentle stream of air over them. Recovery of the internal standard, phenthoate, was monitored as a measure of integrity of the malaoxon analysis and recovery of the acid internal standard, MDA, was determined as a measure of the integrity of the MDA and IDA analyses.

All residue analyses were performed without cleanup on a Tracor MT-220 gas chromatograph equipped with a flame photometric detector operating in the phosphorus mode. The column was a 180 cm x 2 mm ID glass column packed with 3% OV-101 on Gas Chrom Q (100/120 mesh). Operating conditions were: column 175°C, inlet 225°C, detector 240°C; nitrogen 60 ml/min, hydrogen 200 ml/min, oxygen 15 ml/min, and air 50 ml/min.

Urine samples were analyzed as previously described (CHENEY et al. 1975).

RESULTS AND DISCUSSION

Residues found in whole body and organs after exposure of poultry to 75 µg malathion/g are shown in Table I.

Residues of malathion or malaoxon were not found in any body tissue in concentrations greater than 0.10 µg/g after 24 hr exposure to 75 µg malathion/g. However, malathion monoacid and diacid were found in all tissues with the greatest residues in gut and liver. This indicates rapid conversion of malathion to other compounds with the major portion of the malathion being hydrolyzed by carboxylesterase enzyme to monoacid and di-acid (O'BRIEN 1960). Removing malaoxon is the active enzyme inhibitor. The fraction of malathion converted to malaoxon must be small (below 0.10 µg/g) as malaoxon reacts very rapidly to form other compounds and to phosphorylate proteins such as acetylcholinesterase.

Table I

Malathion, malaoxon, malathion monoacid (MDA), and malathion diacid (IDA) residues in poultry after 24 h exposure to 75 µg malathion/g[a]

	Residue (µg/g)			
Organ	Malathion	Malaoxon	MDA	IDA
Whole body	0.[illegible]	ND	4.2	0.38
Brain	ND	ND	1.7	0.22
Liver	ND	ND	4.0	1.25
Fillet	ND	ND	2.5	0.26
Flesh	ND	ND	2.9	0.34
Gut	ND	ND	32.4	3.72

[a]ND indicates less than 0.10 µg/g

The degree of brain acetylcholinesterase inhibition resulting from lethal poisoning of poultry by exposure to 75 µg malathion/g and accumulation of malathion monoacid and di-acid in the gut are shown in Table II.

These data show accumulation of monoacid in the gut that coincides with phosphorylation of acetylcholinesterase in brain and poisoning in short-term continuous exposure to relatively consistent levels of malathion in ingesta. The brain acetylcholinesterase was inhibited 70% after 48% mortality and monoacid in gut had accumulated to 25 µg/g which indicates assessment of monoacid in gut is useful for determining short-term poisoning by malathion.

TABLE II.

Brain acetylcholinesterase (AChE) inhibition and malathion monoacid (MOA) and di-acid (DOA) residues in gut of pinfish exposed to 20 µg malathion/l for 72 h[1]

Hours exposed	Percent killed	AChE[2] activity	Inhibition (%)	Residues (µg/g) MOA	DOA
Control	–	2.72 ± 0.19(27)[3]	–	0	0
0.5	0	1.81 ± 0.23(3)	3	0.05	0.25
1	0	1.85 ± 0.22(3)	6	1.9	0.29
4	0	1.88 ± 0.13(3)	9	2.1	0.48
8	0	1.49 ± 0.34(3)	25[4]	4.8	0.55
24	0	1.80 ± 0.26(3)	35[4]	12.1	0.78
48	0-10	1.65 ± 0.14(3)	46[4]	15.9	1.00
72	60	0.62 ± 0.12(3)	78[4]	18.0	0.92

[1]The measured concentration of malathion in water was 19.9 ± 0.37 µg/l; no residues of malathion or malaoxon (0.10 µg/g) were detected in gut during the test.

[2]AChE activity = µmoles acetylcholine hydrolyzed/hg brain tissue.

[3]Numbers in parentheses indicate replicate samples assayed.

[4]Significantly inhibited at p < 0.001 (Student's t-test).

Data from the exposure of pinfish to 20 µg malathion/l for 24 h followed by 192 h depuration are also in Table III.

Exposure of pinfish to the lower concentration of malathion (10 µg/l) produced correspondingly less brain acetylcholinesterase inhibition and acid residues in gut than the exposure to greater concentration. Only 25% brain acetylcholinesterase inhibition was produced in 24 h and a maximum residue of 7.2 µg monoacid/g of gut was found after 24 h - the greatest residue found. Organic phosphorus declined rapidly to 2.2 µg/g of gut 24 h after exposure was discontinued but brain acetylcholinesterase remained inhibited. Residues and enzyme inhibition were lower 48 h

TABLE III.

Brain acetylcholinesterase (AChE) inhibition and malathion monoacid (MOA) and di-acid (DOA) residues in gut of pinfish exposed to 20 µg malathion/l for 24 h and through 192 h after exposure[1]

Hours		AChE[2]	Inhibition	Residues (µg/g)	
Exposure	Post-exposure	activity	(%)	MOA	DOA
Control	–	2.42 ± 0.14(27)[3]	–	0	0
1	–	1.94 ± 0.15(3)	3	1.2	0.11
4	–	1.88 ± 0.19(3)	6	3.8	0.37
24	–	1.42 ± 0.21(3)	25[4]	7.2	0.72
–	24	1.31 ± 0.15(3)	35[4]	2.2	0.29
–	48	1.65 ± 0.25(3)	28[4]	0.84	0.29
–	120	1.84 ± 0.15(3)	8	0.19	0.09
–	192	–	–	ND	ND

[1]The measured concentration of malathion was 19.8 ± 0.77 µg/l; no residues of malathion or malaoxon (<0.10 µg/g) were detectable in gut during the test.

[2]AChE activity = moles of acetylcholine hydrolyzed/hg brain tissue.

[3]Numbers in parentheses indicate replicate samples assayed.

[4]Significantly inhibited at p <0.001 (Student's t-test)

after exposure was discontinued. However, measurable concentrations of monoacid and di-acid remained in gut 192 h after exposure but brain acetylcholinesterase inhibition had returned to normal range. No residues were found in the gut 192 h after exposure was discontinued.

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